っている。RAF キナーゼ、VEGFR-1-3、PDGFR-βなどを標的とするマルチキナーゼ阻害薬 sorafenib のプラセボコントロールによる無作為化比較試験(SHARP trial)の結果、sorafenib 治療群は無治療群に比べ初めて生存期間の延長が確認された。わが国では肝細胞がんに対する第 I 相試験が行われ、欧米と同じ推奨用量が示されている。今後 sorafenib による化学療法は肝外転移を有する例や局所治療が適応にならない進行例に対する標準治療として位置づけられるものと考えられる。また、肝細胞がんは切除や局所壊死療法など局所治療成功例でも再発率が極めて高いことから、sorafenib は局所治療の補助療法としての適応も期待され、大規模な臨床試験が行われている。さらに現在、より有効な全身治療の確立に向けて、様々な新しい分子標的薬や併用療法の臨床試験が進みつつある。またこれまで sorafenib をはじめ多くの分子標的薬は Child-Pugh A の肝機能が良好な患者でのみ臨床試験が行われてきた。Child-Pugh B 等肝機能低下例での有効性や安全性を検証する研究も必要と考えられる。

E. 結論

肝外転移を有する肝細胞がんの予後は極めて不良であり、有効な全身化学療法の標準治療の確立が必要である。

F. 健康危険情報

特になし

G. 研究発表

論文発表


1. Thomas MB, O'Beirne JP, Furuse J, et al. Systemic Therapy for Hepatocellular Carcinoma: Cytotoxic Chemotherapy, Targeted Therapy and Immunotherapy. Ann Surg Oncol 15:1008-1014, 2008.
2. Ishii H, Furuse J, Kinoshita T,et al. Hepatectomy for hepatocellular carcinoma patients who meet the Milan criteria. Hepatogastroenterology 55:621-626, 2008.
3. Furuse J, Ishii H, Nakachi K,et al. Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. Cancer Sci 99:159-165, 2008.
4. Furuse J. Growth factors as therapeutic targets in HCC. Crit Rev Oncol Hematol 67:8-15, 2008.
5. 古瀬純司，仲地耕平，鈴木英一郎，ほか．肝細胞癌の治療戦略．分子標的治療．消化器外科 31:1017-1023，2008.
6. 古瀬純司．国際共同臨床試験．国際共同臨床試験の現状と課題．肝・胆道・膵がん．腫瘍内科 2:197-204，2008.
7. 古瀬純司．癌の基礎から臨床へ．ベンチからベッドサイドへ．Sorafenib の分子作用機構と肝癌治療．西條長宏(監修)．篠原出版新社，111-117，2008.


学会発表


1. Kudo M, Furuse J, Okusaka T, et al. Phase I/II study of the efficacy and safety of S-1 in patients with advanced hepatocellular carcinoma. 2nd International Liver Cancer Association, 2008, Chicago, USA.
2. FuruseJ, Ikeda M, Okusaka T, et al. A phase II trial of hepatic arterial infusion chemotherapy with cisplatin for advanced hepatocellular carcinoma with portal vein tumor thrombosis. The 33rd ESMO (European Society for Medical Oncology) Congress, 2008. Stockholm


H. 知的財産権の出願・登録状況

(予定を含む)

特許取得:なし

実用新案登録:なし

その他 :なし

Table. Multivariate analysis of prognostic factors in patients with extrahepatic metastasis of hepatocellular carcinoma

| Variables | | Hazard ratio | 95% CI | p-value |
|---|---|---|---|---|
| Performance status | 0 | | | <0.0001 |
| | 1-3 | 2.93 | 2.07-4.29 | |
| Child-Pugh classification | A | | | <0.0001 |
| | B/C | 2.24 | 1.62-3.24 | |
| Metastatic organ | Single | | | 0.0022 |
| | Multiple | 1.77 | 1.23-2.55 | |
| No. of metastatic tumor | Solitary | | | 0.037 |
| | Multiple | 1.72 | 1.03-2.86 | |

# 研究成果の刊行に関する一覧表

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻名 | ページ | 出版年 |
|---|---|---|---|---|---|
| Masuzaki R, Yoshida H, Tateishi R, Shiina S, Omata M. | Hepatocellular carcinoma in viral hepatitis: Imprioving standard therapy. | Best Pract Res Clin Gastroenterology | | In press | 2009 |
| Ohki T, Tateishi R, Shiina S, Goto E, Sato T, Nakagawa H, Masuzaki R, Goto T, Hamamura K, Kanai F, Yoshida H, Kawabe T, Omata M. | Visceral fat accumulation is an independent risk factor of hepatocellular carcinoma recurrence after curative treatment in NASH-suspected patients. | Gut | | In press | 2009 |
| Yamashiki N, Sugawara Y, Tamura S, Kaneko J, Matsui Y, Togashi J, Kokudo N, Omata M, Makuuchi M. | Doube-dose-phase use of second generation heptatitis B virus vaccine in patients after living donor liver transplantation: not an effective measure in transplant recipient. | Hepatol Res | 39 | 7-13 | 2009 |
| Taura N, Yatsuhashi H, Nakao K, Ichikawa T, Ishibashi H. | Long-term trends of the incidence of hepatocellular carcinoma in the Nagasaki prefecture, Japan. | Oncol Rep | 21 | 223-227 | 2009 |
| Migita K, Sawakami-Kobayashi K, Maeda Y, Nakao K, Kondoh S, Sugiura M, Kawasumi R, Segawa O, Tajima H, Machida M, Nakamura M, Yano K, Abiru S, Kawasaki E, Yatsuhashi H, Eguchi K, Ishibashi H. | Interleukin-18 promoter polymorphisms and the disease progression of Hepatitis B virus-related liver disease. | Transl Res | 153 | 91-96 | 2009 |
| Tanaka Y, Kanai F, Tada M, Tateishi R, Sanada M, Nannya Y, Ohta M, Asaoka Y, Seto M, Shiina S, Yoshida H, Kawabe T, Yokosuka O, Ogawa S, Omata M. | Gain of GRLH2 is associated with early recurrence of hepatocellular carcinoma. | J Hepatol | 49 | 746-757 | 2008 |
| Masuzaki R, Tateishi R, Yoshida H, Yoshida H, Sato S, Kato N, Kanai F, Sugioka Y, Ikeda H, Shiina S, Kawabe T, Omata M. | Risk assessment of hepatocellular carcinoma in chronic hepatitis C patients by transient elastography. | J Clin Gastroenterol | 42 | 839-843 | 2008 |
| Yoshida H, Yoshida H, Goto E, Sato T, Ohki T, Masuzaki R, Tateishi R, Goto T, Shiina S, Kawabe T, Omata M. | Safety and efficacy of lamivudine after radiofrequency ablation in patients with hepatitis B virus-related hepatocellular carcinoma. | Hepatol Int | 2 | 89-94 | 2008 |
| Tateishi R, Yoshida H, Matsuyama Y, Mine N, Kondo Y, Omata M. | Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. | Hepatol Int | 2 | 17-30 | 2008 |
| Ohki T, Tateishi R, Sato T, Masuzaki R, Imamura J, Goto T, Yamashiki N, Yoshida H, Kanai F, Kato N, Shiina S, Yoshida H, Kawabe T, Omata M. | Obesity is an independent risk factor for hepatocellular carcinoma development in chronic hepatitis patients. | Clin Gastroenterol H | 6 | 459-464 | 2008 |
| Kondo Y, Yoshida H, Tateishi R, Shiina S, Kawabe T, Omata M. | Percutaneous radiofrequency ablation of liver cancer in the hepatic dome with intrapleural fluid infusion technique. | Br J Surg | 95 | 996-1004 | 2008 |
| Masuzaki R, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Goto T, Yoshida H, Kanai F, Sugioka Y, Ikeda H, Shiina S, Kawabe T, Omata M. | Comparison of liver biopsy and transient elastography based on clinical relevance. | Can J Gastroenterol | 22 | 753-757 | 2008 |

| | | | | | |
|---|---|---|---|---|---|
| Imamura J, Tatcishi R, Shiina S, Goto E, Sato T, Ohki T, Masuzaki R, Goto T, Yoshida H, Kanai F, Hamamura K, Obi S, Yoshida H, Omata M. | Neoplastic seeding after radiofrequency ablation for hepatocellular carcinoma. | Am J Gastroenterol | 103 | 3057-3062 | 2008 |
| Kanda M, Tateishi R, Yoshida H, Sato T, Masuzaki R, Ohki T, Imamura J, Goto T, Yoshida H, Hamamura K, Obi S, Kanai F, Shiina S, Omata M. | Extrahepatic metastasis of hepatocellular carcinoma -incidence and risk factors-. | Liver Int | 28 | 1256-1263 | 2008 |
| Masuzaki R, Omata M. | Treatment of hepatocellular carcinoma. | Ind J Gastroenterol | 27 | 113-122 | 2008 |
| Thomas MB, O'Beirne JP, Furuse J, et al. | Systemic therapy for hepatocellular carcinoma: cytotoxic chemotherapy, targeted therapy and immunotherapy. | Ann Surg Oncol | 15 | 1008-1014 | 2008 |
| Ishii H, Furuse J, Kinoshita T,et al. | Hepatectomy for hepatocellular carcinoma patients who meet the Milan criteria. | Hepatogastroenterology | 55 | 621-626 | 2008 |
| Furuse J, Ishii H, Nakachi K,et al. | Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. | Cancer Sci | 99 | 159-165 | 2008 |
| Furuse J. | Growth factors as therapeutic targets in HCC. | Crit Rev Oncol Hematol | 67 | 8-15 | 2008 |
| 古瀬純司, 仲地耕平, 鈴木英一郎,ほか. | 肝細胞癌の治療戦略. 分子標的治療 | 消化器外科 | 31 | 1017-1023 | 2008 |
| 古瀬純司 | 国際共同臨床試験. 国際共同臨床試験の現状と課題. 肝・胆道・膵がん. | 腫瘍内科 | 2 | 197-204 | 2008 |
| 古瀬純司 | Sorafenibの分子作用機構と肝癌治療 | 癌の基礎から臨床へ. ベンチからベッドサイドへ西條長宏(監修) 篠原出版新社 | | 111-117 | 2008 |

*Hepatology Research* 2009; 39: 7–13 doi: 10.1111/j.1872-034X.2008.00412.x

*Original Article*

# Double-dose double-phase use of second generation hepatitis B virus vaccine in patients after living donor liver transplantation: Not an effective measure in transplant recipients

Noriyo Yamashiki,[1,3] Yasuhiko Sugawara,[1,2] Sumihito Tamura,[2] Junichi Kaneko,[2] Yuichi Matsui,[2] Junichi Togashi,[2] Norihiro Kokudo,[1,2] Masao Omata[3] and Masatoshi Makuuchi[1,2,4]

[1]*Organ Transplantation Service,* [2]*The Artificial Organ and Transplantation Division, Department of Surgery,* [3]*Department of Gastroenterology, University of Tokyo, Graduate School of Medicine, and* [4]*Department of Surgery, Red Cross Medical Center, Tokyo, Japan*

*Aims:* Post-transplant active immunization for chronic hepatitis B patients has been attempted in several studies with controversial results. We assessed the effect of a double-dose double-phase vaccination regimen among partial living donor liver recipients.

*Methods:* Eighteen patients who underwent liver transplantation (LT) for chronic hepatitis B and two non-hepatitis B virus (HBV)-infected patients who received hepatitis B core antibody (HBcAb)-positive donor organs were recruited 18–78 months after LT. All were on hepatitis B immunoglobulin (HBIG) mono-prophylaxis before and throughout vaccination, to maintain hepatitis B surface antibody (HBsAb) titers of more than 100 IU/mL. Recombinant hepatitis B surface antigen vaccine (40 μg) was administered intramuscularly during weeks 0, 4, 8, 24, 28 and 32.

*Results:* The patients consisted of 15 males and five females with a median age of 52 (39–59) years. None developed a sufficient HBsAb titer above 500 IU/mL by week 48. In two patients whose maximum HBsAb titer increased to above 300 IU/mL, we attempted to skip HBIG, but shortly thereafter the titer dropped below 100 IU/mL and HBIG administration was resumed. Although the HBIG dose was reduced during and after vaccination, cessation of administration was not achieved.

*Conclusion:* Double-dose double-phase use of second generation recombinant vaccine was not effective in this study population. The selected population should be targeted for a conventional vaccine regimen, and different approaches, such as strong adjuvant or pre-S containing protein, should be further tested in a larger number of patients after LT for chronic hepatitis B.

**Key words:** Hepatitis B vaccine, HBcAb positive donor, HBIG, lamivudine, liver transplantation, prophylaxis

## INTRODUCTION

THE LONG-TERM use of hepatitis B immunoglobulin (HBIG) and/or nucleos(t)ide analog prophylaxis has dramatically improved survival rates after liver transplantation for hepatitis B virus (HBV)-related liver disease.[1] Historically, hepatitis B (HB) recurs in approximately 80% of liver transplant recipients with HBV-related liver diseases. The use of HBIG mono-prophylaxis has improved the rate of recurrent hepatitis to 35%.[7] Long-term use of HBIG therapy, however, is costly and there is insufficient evidence regarding the optimal length of administration. Lamivudine (LAM) mono-prophylaxis is less costly and is useful for decreasing the rate of hepatitis recurrence to less than 40%.[3,4] Emerging resistant strains are a concern in long-term follow-up for transplant recipients, however, and additional nucleos(t)ide analogs such as adefovir and entecavir are required.[5,6] The combination of HBIG and antiviral agents has decreased the rate of hepatitis B recurrence.[7,8]

*Correspondence: Dr Yasuhiko Sugawara, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: yasusuga-tky@umin.ac.jp*
*Received 29 March 2008; revision 7 July 2008; accepted 8 July 2008.*

Active immunization against hepatitis surface antigens has been attempted in patients after liver transplantation for HB-related liver diseases.[9–11] Repeated vaccination effectively initiates the production of anti-hepatitis B antibodies and is thus followed by HBIG administration withdrawal.[9–11] This idea is theoretically a more economical and simple method compared to passive immunization or nucleos(t)ide analog administration. Contradictory results have been reported, however, and suitable candidates for this type of vaccination have not been determined.[12–15]

At the University of Tokyo, we use HBIG monoprophylaxis.[16] The ultimate goal of vaccination is to achieve sufficient production of anti-hepatitis B immunoglobulin and to discontinue further prophylaxis against recurrent hepatitis B. In the present study, we report the results of an active immunization protocol in chronic hepatitis B-related living donor liver transplantation (LDLT) recipients.

## METHODS

### Subject selection

PATIENTS WHO UNDERWENT LDLT at least 18 months before for HBV-related end-stage liver disease or received hepatitis B core antibody (HBcAb)-positive donor livers were enrolled if they were being treated with an HBIG prophylaxis protocol, free of nucleos(t)ide analogs, without co-infection with hepatitis C virus or human immunodeficiency virus, and if they had no evidence of HBV reactivation. Twenty Japanese patients provided informed consent to the study protocol. There were 15 men and five women with a median age of 52, ranging from 39 to 59 years. Etiologies of end-stage liver disease included chronic hepatitis B in 16, fulminant hepatic failure with a history of chronic hepatitis B in two, and end-stage liver disease due to autoimmune hepatitis and primary biliary cirrhosis in one each. Two non-HBV patients and one chronic hepatitis B patient received core antibody-positive donor organs. Nine patients with hepatocellular carcinoma were free from post-transplant recurrence with a median follow-up period of 43 (18–90) months.

The donors were 13 men and seven women ranging in age from 18 to 54 years and weighing 43–75 kg. Their relationship to the patients included 10 children, five spouses, three nephews, one sibling and one cousin. Right liver graft was performed in eight, extended right graft in one, right lateral graft in two, left lobe with caudate graft in seven and left lobe graft in two. Three donors were positive for both HB surface antibody (HBsAb) and HBcAb.

### Pre- and post-LDLT follow-up protocol

The post-transplantation immunosuppression regimen consisted of steroid induction with tacrolimus, or cyclosporine in case of tacrolimus intolerance, for maintenance.[17] Among the 18 patients with hepatitis B virus infection, LAM 100 mg/day was given orally prior to LDLT. One patient received LAM for more than 1 year, one for 3 months, and 15 received LAM for less than 4 weeks. Of the 17 patients on LAM, a negative HBV-DNA load was confirmed preoperatively in nine cases. In eight patients whose HBV-DNA was detectable pretransplant, LAM therapy was continued for 4 weeks after LDLT, and discontinued after confirming negative HBV-DNA. Postoperatively, HBIG (Mitsubishi Tanabe Pharma, Hebsbulin-IH, Tokyo, Japan) was administered to HBV-infected patients and those who received HBcAb-positive donor organs. Details of the HBIG administration protocol and doses are described elsewhere.[16] In brief, HBIG was administered to maintain the anti-HB surface antibody (HbsAb) levels at greater than 1000 IU/L for patients with HBV and greater than 500 IU/L for patients that received HBcAb-positive donor organs. After 1 year, 1000–2000 U was given intravenously indefinitely to maintain HbsAb levels of greater than 100 IU/L.[16,18]

### Vaccination protocol

After obtaining informed consent, baseline laboratory tests were performed in all patients. Table 1 shows the baseline patient characteristics and laboratory findings. HBV-DNA was undetectable in all patients. Increased dose, namely "double-dose", of recombinant anti-HB vaccination (40 μg/2 mL; Heptavax II, Banyu Pharm, Tokyo, Japan) was injected bilaterally into the deltoid muscles (half dose each side). HBsAb titers were measured at week 0 and every 4 weeks thereafter. Vaccination was scheduled for two phases of three administration cycles at weeks 0, 4, 8, 24, 28 and 32.

During the protocol period, HBIG was administered 2 weeks before and after vaccination if necessary; either 1000 or 2000 IU was administered intravenously according to the previously measured HBsAb titer, as long as it was maintained above 100 IU/mL.

### Study end-points and ethical considerations

The primary end-point of this study was the vaccine response. A significant increase, that is >500 IU/mL, in

**Table 1** Patient characteristics

| Factors | Values[1] |
|---|---|
| Age | 52 (39–59) years |
| Men, women | 15, 5 |
| HBV-related hepatitis | 18 |
| HBcAb positive donor | 3 |
| Immunosuppression (Tac + CS), (CyA + CS) | 15, 5 |
| Months from LDLT (median) | 45 (17–90) months |
| Pre-LDLT LAM | 17 |
| Duration of LAM administration before LDLT: <1 month, 1–12 months, >12 months | 15, 1, 1 |
| HBV-DNA positive at LDLT[2] | 8 |
| HBsAg titer at entry | 138.5 (92–302) IU/mL |
| Aspartate aminotransferase | 18.5 (7–30) IU/mL |
| Alanine aminotransferase | 16 (5–30) IU/mL |
| Alkaline phosphatase | 197 (103–528) IU/mL |
| γ-glutamyl transferase | 33.5 (11–187) IU/mL |
| Lactate dehydrogenase | 178 (99–315) IU/mL |
| Total bilirubin | 0.9 (0.1–1.5) mg/dl |

[1]Values are number or median (range). [2]HBV-DNA measured by transcription-mediated amplification methods. Lower detectable limit is 3.7 LEG/mL.
CS, corticosteroid; CyA, cyclosporine; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; HBV, hepatitis B virus; LAM, lamivudine; LDLT, living donor liver transplantation; Tac, tacrolimus.

HBsAb 12 weeks after the last vaccination was considered effective. Secondary end-points were changes in the required HBIG dose.

The study protocol was approved by the institutional review board (No. P2005015-11X) and informed consent was obtained from each patient.

### Statistical analysis

The number of units of HBIG administered before and during the study protocol was recorded, and the sum of the number of HBIG units administered at −24 to −1 weeks, 0–23 weeks, 24–47 weeks and 48–71 weeks was compared by the Friedman test.

## RESULTS

ALL PATIENTS COMPLETED the full vaccination course. Double-dose vaccination was well-tolerated with the only side effect of local pain not requiring analgesics. During the study period, none of the cases developed active hepatitis or rejection. One patient developed kidney dysfunction during the study and was unable to complete the HBIG administration protocol (case #5).

Pre-, post- and maximum serum HBsAb titers are shown in Table 2. None of the cases developed a sufficient HBsAb titer level of >500 IU/mL (Fig. 1). None achieved cessation of HBIG administration.

Three cases (#2, #3, #20) developed maximum titers of 313, 408 and 469 IU/mL, and HBIG was thus discontinued in two of them (#2 and #3). The titers then decreased below 100 IU/mL in both cases, however, and HBIG administration was resumed (Fig. 2). Case #20 desired to continue HBIG administration despite the elevation of HBsAb titer to 469 IU/mL. Eventually, HBsAb titer dropped to 214 IU/mL and thus the vaccination was considered ineffective.

HBIG administration in four 24-week periods (−24 to −1, 0 to 23, 24 to 47, 48 to 71) in each case are shown in Table 2. In case 11, HBIG administration between weeks 48 and 71 was stopped due to the appearance of HBsAg and HBV-DNA. HBIG doses as a whole, excluding case 11, decreased over time ($P = 0.006$), as illustrated by the box plot (Fig. 3).

After the final vaccination, all patients were followed for a median of 17 (10–18) months. All patients are alive. In two cases (#11 and #13), serum HBsAg and HBV-DNA were observed at 4 and 8 months after the vaccination protocol, and 60 and 58 months after liver transplantation. In these two cases, the minimum HBsAb level was above 100 IU/mL even after our vaccination protocol. These cases are currently on antiviral therapy and are free from signs of active hepatitis.

## DISCUSSION

ACTIVE IMMUNIZATION FOR chronic hepatitis B has been attempted in patients after liver transplantation. In early studies, commercially available recombinant vaccine was used in patients receiving HBIG mono-prophylaxis and was effective in 64–80% of patients.[9,10] This immune response was sustained for a longer follow-up period of 41 (31–85) months in 14 responders.[10] The use of a potential adjuvant in combination with recombinant vaccine is remarkably effective in 80% of post-transplant recipients, and the HBsAb titer was maintained for a long (8–27 months) follow-up period.[11] Studies by other groups, however, demonstrated unfavorable results in patients who were either on LAM prophylaxis or HBIG mono-prophylaxis.[12–14] Several factors are thought to

Table 2 Characteristics, HBsAb titer and dose requirement of HBIG

| Case | Age | Sex | Etiology | Donor HBcAb | Pre-LT HBV-DNA | Pre-LT LAM | Timing of vaccination[1] | IS | Pre-HBsAb[1] | Max HBsAb[2] | End HBsAb[1] | HBIG administration | | | | Outcome[1] (recurrence[4]) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | −24–1 weeks[3] | 48–71 weeks[3] | 0–23 weeks[3] | 24–47 weeks[3] | |
| 1 | 52 | F | PBC | + | Negative | NA | 78 | Tac | 165 | 253 | 253 | 10 | 7 | 10 | 11 | 104, alive |
| 2 | 46 | F | AIH | + | Negative | NA | 51 | CyA | 92 | 313 | 152 | 3 | 5 | 7 | 3 | 75, alive |
| 3 | 54 | M | B-FHF | − | Positive | Yes | 48 | Tac | 122 | 408 | 136 | 12 | 5 | 8 | 5 | 65, alive |
| 4 | 39 | M | B-FHF | − | Positive | Yes | 30 | Tac | 163 | 227 | 221 | 8 | 6 | 6 | 5 | 56, alive |
| 5 | 50 | M | BLC, HCC | + | Negative | Yes | 90 | Tac | 175 | 79 | 48 | 6 | 5 | 6 | 7 | 115, alive |
| 6 | 57 | F | BLC | − | Positive | Yes | 57 | Tac | 127 | 191 | 107 | 7 | 6 | 9 | 7 | 82, alive |
| 7 | 47 | F | BLC | − | Negative | Yes | 56 | Tac | 140 | 186 | 132 | 7 | 6 | 6 | 6 | 81, alive |
| 8 | 50 | M | BLC, HCC | − | Negative | Yes | 55 | Tac | 101 | 153 | 113 | 5 | 6 | 5 | 4 | 77, alive |
| 9 | 50 | M | BLC, | − | Positive | Yes | 51 | Tac | 117 | 114 | 70 | 7 | 6 | 6 | 6 | 75, alive |
| 10 | 51 | M | BLC, HCC | − | Positive | Yes | 49 | Tac | 111 | 293 | 138 | 8 | 5 | 6 | 5 | 74, alive |
| 11 | 48 | M | BLC, HCC | − | Positive | Yes | 48 | Tac | 281 | 171 | 131 | 11 | 1 | 10 | 4 | 71, alive, rec 60 |
| 12 | 59 | M | BLC | − | Positive | Yes | 43 | CyA | 160 | 176 | 162 | 6 | 8 | 6 | 6 | 69, alive |
| 13 | 52 | M | BLC, HCC | − | Positive | Yes | 43 | Tac | 209 | 210 | 132 | 8 | 7 | 6 | 6 | 67, alive, rec 56 |
| 14 | 57 | F | BLC, HCC | − | Negative | Yes | 41 | CyA | 109 | 220 | 220 | 12 | 7 | 10 | 8 | 67, alive |
| 15 | 56 | M | BLC, HCC | − | Negative | None | 40 | Tac | 144 | 188 | 93 | 9 | 10 | 12 | 12 | 60, alive |
| 16 | 54 | M | BLC | − | Negative | Yes | 40 | Tac | 137 | 190 | 190 | 8 | 6 | 5 | 5 | 60, alive |
| 17 | 50 | M | BLC | − | Negative | Yes | 20 | CyA | 95 | 262 | 140 | 6 | 6 | 6 | 6 | 38, alive |
| 18 | 51 | M | BLC, HCC | − | Negative | Yes | 19 | CyA | 135 | 179 | 115 | 11 | 8 | 10 | 9 | 44, alive |
| 19 | 52 | M | BLC, HCC | − | Negative | Yes | 18 | Tac | 152 | 245 | 121 | 14 | 7 | 12 | 6 | 43, alive |
| 20 | 53 | M | BLC | − | Negative | Yes | 17 | Tac | 302 | 469 | 214 | 14 | 10 | 12 | 12 | 37, alive |

[1]Number indicates months after liver transplantation. [2]IU/mL. [3]Number indicates vials of HBIG (1V = 1000 Units) used during the period. [4]Number indicates months after liver transplantation, when HBV-DNA became positive.

AIH, autoimmune hepatitis; B-FHF, hepatitis B related fulminant hepatic failure; BLC, hepatitis B related liver cirrhosis; CyA, cyclosporine A; FHF, fulminant hepatic failure; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; HBIG, hepatitis B immunoglobulin; HCC, hepatocellular carcinoma; IS, immunosuppression; LAM, lamivudine; LC, liver cirrhosis; LT, liver transplantation; PBC, primary biliary cirrhosis; Tac, tacrolimus.

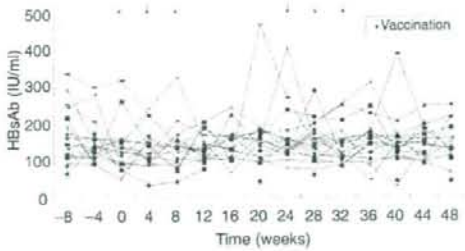


**Figure 1** Change in serum hepatitis B surface antibody (HBsAb) titers before and during the vaccination protocol. Heptavax II (40 μg) was administered at weeks 0, 4, 8, 24, 28 and 32. HBsAb levels were between 100 IU/mL and 200 IU/mL in most cases. There was no significant response to vaccination throughout the study.

contribute to a good response, such as younger age, use of an adjuvant to the vaccine and negative HBV-DNA preoperatively. Use of LAM was once speculated to have a negative effect, and, in another study, Angelico *et al.* failed to reveal a favorable effect of mono-prophylaxis with HBIG.[12]

In our study, the double-dose double-phase use of a second generation recombinant hepatitis B vaccine was tested. Our institution applies an HBIG mono-prophylaxis protocol against hepatitis B recurrence. For non-replicate HBV disease, LAM is discontinued at the time of transplantation. During this study, HBIG was administered throughout the vaccination protocol according to Binzele's report,[11] and anti-hepatitis B antibody levels of greater than 500 IU/mL were determined to be effective. Unfortunately, none of the 20 patients developed an adequate anti-hepatitis B antibody titer after two vaccination cycles. Although the dose of HBIG administration decreased during and after the vaccination protocol, we did not consider it a significant change, since none achieved cessation of HBIG administration. The possible factors contributing to vaccination failure among the subjects are that 15 of the 20 patients were aged 50 years or older, eight of 18 chronic hepatitis B patients had a HBV replicative state at the

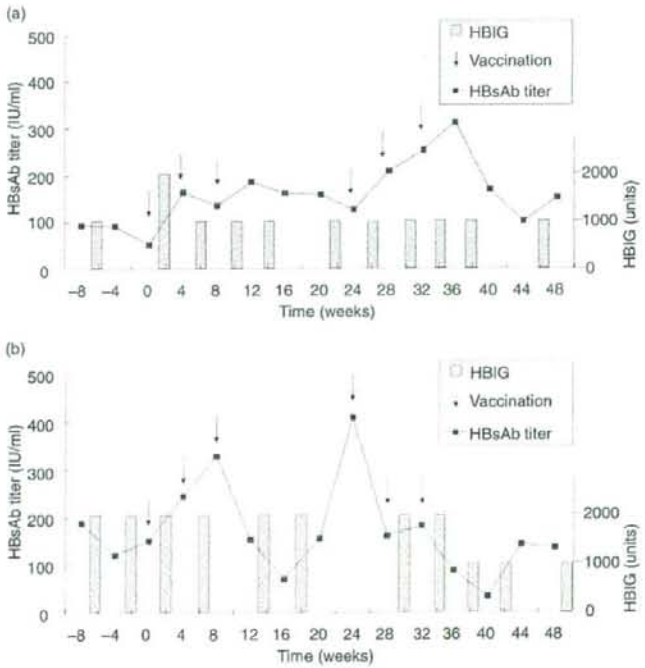


**Figure 2** Hepatitis B surface antibody (HBsAb) titers in response to vaccination. (a) Case 2: 46-year-old female with auto-immune hepatitis (AIH), hepatitis B core antibody (HBcAb)-positive donor. (b) Case 3: 54 year-old male with B-fulminant hepatic failure. Hepatitis B immunoglobulin (HBIG) administration was skipped in response to elevated HBsAb level. The HBsAb titer then dropped and HBIG administration was resumed in both cases.

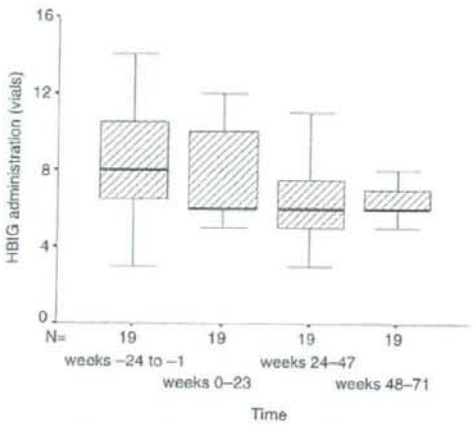


Figure 3 Hepatitis B immunoglobulin (HBIG) administration in four 24-week periods (−24 to −1, 0–23, 24–47, 48–71) is graphically shown by box plot. The dose of HBIG administration decreased over the time period ($P = 0.006$). Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, 90th percentile; lower bar outside box, 10th percentile.

time of transplantation, use of corticosteroids in combination with a calcineurine inhibitor, and one patient was receiving hemodialysis.[20] These factors might have affected the results of this study negatively.

A second generation recombinant HB vaccine is used for prophylaxis in the healthy population. HBsAg-specific T and B cells are induced and a sufficient amount of HBsAb can be produced to neutralize circulating HB virus particles. The recombinant HB vaccines containing S, pre-S2 and/or pre-S1, the so-called the third generation vaccines, have immunogenic advantages over the second generation recombinant HB vaccines; they more efficiently induce an immune response than the second generation HB vaccines among a healthy population,[21,22] and induce not only anti-S antibodies, but also anti-pre-S2 antibodies. Such a vaccine containing S, pre-S1 and pre-S2 antigens was used among Chinese patients who underwent liver transplantation for chronic HB and were on LAM prophylaxis.[23] The authors of that study reported that the vaccine was effective in 10 of 20 patients. An earlier study by Karasu *et al.*,[13] however, failed to show the effectiveness of pre-S1, pre-S2 and S gene products.

Prevention of hepatitis B infection is equally important in HBsAg-negative patients receiving HBc-positive donor organs, as the risk for de novo HB is high.[24] The current standard of care for transplant recipients of HBcAb-positive donor organs is similar to that for patients with chronic HB, including long-term HBIG and/or nucleos(t)ide analog.[16,24–26] In our study, HB vaccine was administered to two HBV non-infected patients who received HBcAb-positive donor organs. Neither of these two patients, however, responded. Pediatric transplantation recipients who receive prior vaccination under an immunization program are likely to achieve a high anti-HB titer by active immunization after LDLT.[27] LAM prophylaxis was withdrawn after 2 years if an adequate anti-hepatitis B titer was achieved. Soejima *et al.*[28] recently reported that 6 of 11 Japanese patients receiving liver transplants for fulminant hepatitis B or for non-HBV diseases with HBcAb-positive donor organs had seroconversion. The tested patients were younger, with a median age of 33 years. These patients were on combination prophylaxis with LAM and HBIG, and HBIG was withdrawn after vaccination. The question remains, however, as to whether LAM therapy can be discontinued in the future among this population.

Vaccination may be promising in selected populations, such as in younger recipients or in those with fulminant hepatitis or HBsAg-negative recipients receiving HBcAb-positive donor organs. For patients older than 50 with vaccination failure or chronic hepatitis B patients, a different approach may prove optimal, such as the use of a pre-S containing vaccination. The research findings for the use of such vaccines are controversial, however, warranting further study.

## ACKNOWLEDGMENTS

THIS WORK WAS supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Grants-in-Aid for Research on HIV/AIDS and Research on Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan.

## REFERENCES

1 Samuel D, Muller R, Alexander G *et al.* Liver transplantation in European patients with the hepatitis B surface antigen. *N Engl J Med* 1993; 329: 1842–7.

2 Todo S, Demetris AJ, Van Thiel D, Teperman L, Fung JJ, Starzl TE. Orthotopic liver transplantation for patients with hepatitis B virus-related liver disease. *Hepatology* 1991; **13**: 619–26.
3 Shouval D, Samuel D. Hepatitis B immune globulin to prevent hepatitis B virus graft reinfection following liver transplantation: a concise review. *Hepatology* 2000; **32**: 1189–95.
4 Mutimer D, Dusheiko G, Barrett C *et al.* Lamivudine without HBIg for prevention of graft reinfection by hepatitis B: long-term follow-up. *Transplantation* 2000; **70**: 809–15.
5 Perrillo RP, Wright T, Rakela J *et al.* A multicenter United States–Canadian trial to assess lamivudine monotherapy before and after liver transplantation for chronic hepatitis B. *Hepatology* 2001; **33**: 424–32.
6 Lo CM, Cheung ST, Lai CL *et al.* Liver transplantation in Asian patients with chronic hepatitis B using lamivudine prophylaxis. *Ann Surg* 2001; **233**: 276–81.
7 Rosenau J, Tillmann HL, Bahr MJ *et al.* Successful hepatitis B reinfection prophylaxis with lamivudine and hepatitis B immune globulin in patients with positive HBV-DNA at time of liver transplantation. *Transpl Proc* 2001; **33**: 3637–8.
8 Gane EJ, Angus PW, Strasser S *et al.* Lamivudine plus low-dose hepatitis B immunoglobulin to prevent recurrent hepatitis B following liver transplantation. *Gastroenterology* 2007; **132**: 931–7.
9 Sanchez-Fueyo A, Rimola A, Grande L *et al.* Hepatitis B immunoglobulin discontinuation followed by hepatitis B virus vaccination: a new strategy in the prophylaxis of hepatitis B virus recurrence after liver transplantation. *Hepatology* 2000; **31**: 496–501.
10 Sanchez-Fueyo A, Martinez-Bauer E, Rimola A Hepatitis B vaccination after liver transplantation. *Hepatology* 2002; **36**: 257–8.
11 Bienzle U, Gunther M, Neuhaus R, Neuhaus P. Successful hepatitis B vaccination in patients who underwent transplantation for hepatitis B virus-related cirrhosis: preliminary results. *Liver Transpl* 2002; **8**: 562–4.
12 Angelico M, Di Paolo D, Trinito MO *et al.* Failure of a reinforced triple course of hepatitis B vaccination in patients transplanted for HBV-related cirrhosis. *Hepatology* 2002; **35**: 176–181.
13 Karasu Z, Ozacar T, Akarca U *et al.* HBV vaccination in liver transplant recipients: not an effective strategy in the prophylaxis of HBV recurrence. *J Viral Hepat* 2005; **12**: 212–215.
14 Lo CM, Liu CL, Chan SC, Lau GK, Fan ST. Failure of hepatitis B vaccination in patients receiving lamivudine prophylaxis after liver transplantation for chronic hepatitis B. *J Hepatol* 2005; **43**: 283–7.
15 Rosenau J, Hooman N, Hadem J *et al.* Failure of hepatitis B vaccination with conventional HBsAg vaccine in patients with continuous HBIG prophylaxis after liver transplantation. *Liver Transpl* 2007; **13**: 367–73.
16 Sugawara Y, Makuuchi M, Kaneko J, Akamatsu N, Imamura H, Kokudo N. Living donor liver transplantation for hepatitis B cirrhosis. *Liver Transpl* 2003; **9**: 1181–4.
17 Sugawara Y, Makuuchi M, Kaneko J, Ohkubo T, Imamura H, Kawarasaki H. Correlation between optimal tacrolimus doses and the graft weight in living donor liver transplantation. *Clin Transpl* 2002; **16**: 102–6.
18 Takemura N, Sugawara Y, Tamura S, Makuuchi M. Liver transplantation using hepatitis B core antibody-positive grafts: review and university of Tokyo experience. *Dig Dis Sci* 2007; **52**: 2472–7.
19 Albeniz Arbizu E, Barcena Marugan R, Oton Nieto E *et al.* Prophylaxis of recurrent hepatitis B virus by vaccination after liver transplant: preliminary results. *Transpl Proc* 2003; **35**: 1848–9.
20 Stevens CE, Alter HJ, Taylor PE, Zang EA, Harley EJ, Szmuness W Hepatitis B vaccine in patients receiving hemodialysis. Immunogenicity and efficacy. *N Engl J Med* 1984; **311**: 496–501.
21 Shapira MY, Zeira E, Adler R, Shouval D. Rapid seroprotection against hepatitis B following the first dose of Pre-S1/Pre-S2/S vaccine. *J Hepatol* 2001; **34**: 123–7.
22 Zucherman JN, Zucherman AJ, Symington I *et al.* Evaluation of a new hepatitis B triple-antigen vaccine in inadequate responders to current vaccines. *Hepatology* 2001; **34**: 798–802.
23 Lo CM, Lau GK, Chan SC, Fan ST, Wong J. Efficacy of a pre-S containing vaccine in patients receiving Lamivudine prophylaxis after liver transplantation for chronic hepatitis B. *Am J Transpl* 2007; **7**: 434–9.
24 de Villa VH, Chen YS, Chen CL Hepatitis B core antibody-positive grafts: recipient's risk. *Transplantation* 2003; **75**: S49–53.
25 Barcena Marugan R, Garcia-Hoz F, Vazquez Romero M *et al.* Prevention of de novo hepatitis B infection in liver allograft recipients with previous hepatitis B infection or hepatitis B vaccination. *Am J Gastroenterol* 2002; **97**: 2398–401.
26 Bienzle U, Gunther M, Neuhaus R *et al.* Immunization with an adjuvant hepatitis B vaccine after liver transplantation for hepatitis B-related disease. *Hepatology* 2003; **38**: 811–19.
27 Lin CC, Chen CL, Concejero A *et al.* Active immunization to prevent de novo hepatitis B virus infection in pediatric live donor liver recipients. *Am J Transpl* 2007; **7**: 195–200.
28 Soejima Y, Ikegami T, Taketomi A *et al.* Hepatitis B vaccination after living donor liver transplantation. *Liver Int* 2007; **27**: 977–82.

# Long-term trends of the incidence of hepatocellular carcinoma in the Nagasaki prefecture, Japan

NAOTA TAURA[1], HIROSHI YATSUHASHI[1], KAZUHIKO NAKAO[2], TATSUKI ICHIKAWA[2] and HIROMI ISHIBASHI[1]

[1]Clinical Research Center, National Nagasaki Medical Center, Kubara 2-1001-1, Omura, Nagasaki 856-8562; [2]The First Department of Internal Medicine, Nagasaki University School of Medicine, Sakamoto 1-7-1, Nagasaki 852-8501, Japan

Received April 14, 2008; Accepted August 4, 2008

DOI: 10.3892/or_00000212

**Abstract.** The incidence of hepatocellular carcinoma (HCC) in Japan is still increasing. The aim of the present study was to analyze the epidemiological trend of HCC in the Western area of Japan, Nagasaki. A total of 1,807 patients with HCC diagnosed at our two hospitals between 1981 and 2005 were consecutively recruited for this study. Cohorts of patients with HCC were categorized into five-year intervals. The etiology of HCC was categorized into four groups: HCC-B: HBsAg positive, HCVAb negative, HCC-C: HCVAb positive, HBsAg negative, HCC-BC: both of HBsAg and HCVAb positive and HCC-nonBC: both of HBsAg and HCVAb negative. The number and proportion of HCC-B cases decreased from 1986 to 1990 and thereafter stabilized, whereas those of HCC-C reached the peak from 1995 to 2000 and thereafter decreased. On the other hand, the number and ratio of the HCC-nonBC cases continued to increase in the whole period. The male/female ratio of HCC-C patients decreased from 6.4 in the period 1981-1985 to 1.9 in 2001-2005, indicating clearly the increase of female patients. On the other hand, the male/female ratio of other types of HCC patients did not change during the period. HCC patients rapidly increased from 1981 to 2000 and this increase was originated from that of HCC-C. The increase of the median age and the number of female patients with HCC-C was also demonstrated. The increase in the number and the proportion of the HCC-nonBC patients was also significant.

## Introduction

Primary liver cancer is the most common primary cancer of the liver accounting for ~6% of all human cancers. It is estimated that half a million cases occur worldwide annually, making primary liver cancer the fifth most common malignancy in men and the ninth in women (1-6). Hepatocellular carcinoma (HCC) accounts for 85 to 90% of primary liver cancers (7) and the age-adjusted HCC mortality rate has increased in recent decades in Japan (8). Similarly, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia (9,10). HCC often develops in patients with liver cirrhosis caused by hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption, or nonalcoholic fatty liver disease. Of the hepatitis viruses which cause HCC, HCV is predominant in Japan (11-14).

Although the age-adjusted incidence of HCC has increased in Japan, sequential changes in background features of HCC patients are not fully understood (15). Yoshizawa reports that deaths due to HCC in Japan have continued to increase in males, particularly in those older than 60 years of age in the past 3 decades, although the reasons for this are unclear (16). To clarify factors affecting epidemiological changes in Japanese HCC patients, especially the change in age distribution and gender, we analyzed the underlying features of HCC patients in a two major liver center-based study.

## Patients and methods

*Patients.* A total of 1,807 patients with HCC diagnosed between January 1981 and December 2005 in the Liver Disease Center, National Nagasaki Medical Center and in the outpatient clinic of The First Department of Internal Medicine, Nagasaki University Hospital, were consecutively recruited for this study. The diagnosis of HCC was based on AFP levels and imaging techniques including ultrasonography (USG), computerized tomography (CT), magnetic resonance imaging (MRI), hepatic angiography (HAG) and/or tumor biopsy. The diagnostic criteria for HCC were either a confirmative tumor biopsy or elevated AFP (≥20 ng/ml) and neovascularization in HAG and/or CT. Cohorts of patients with HCC were categorized into five-year intervals (1981-1985, 1986-1990, 1991-1995, 1996-2000 and 2001-2005).

*Etiology of HCC.* Sera were stored at -80˚C until use. A diagnosis of chronic HCV infection was based on the presence of HCVAb (microparticle enzyme immunoassay; Abbott

*Correspondence to*: Dr Naota Taura, Clinical Research Center, National Nagasaki Medical Center, Kubara 2-1001-1, Omura, Nagasaki 856-8562, Japan
E-mail: ntaura@nmc-research.jp

*Key words:* hepatitis C virus, hepatocellular carcinoma, aging, Japan

Table I. The characteristics of HCC patients, 1981-2005.

| Period | 1981-1985 | 1986-1990 | 1991-1995 | 1996-2000 | 2001-2005 | Total |
|---|---|---|---|---|---|---|
| Number | 240 | 316 | 369 | 419 | 463 | 1807 |
| Gender | | | | | | |
| Male | 194 | 257 | 268 | 314 | 314 | 1347 |
| Female | 46 | 59 | 101 | 105 | 149 | 460 |
| Ratio (male/female) | 4.2 | 4.4 | 2.7 | 3.0 | 2.1 | 2.9 |
| Age (y.o) (IQR) | 57 (6.5) | 61 (5.1) | 63 (5.4) | 66 (5.1) | 68 (6.3) | 64 (6.5) |
| Hepatitis virus | | | | | | |
| HCC-B | 95 | 70 | 80 | 67 | 100 | 412 |
| HCC-C | 111 | 213 | 240 | 292 | 278 | 1134 |
| HCC-B+C | 8 | 8 | 9 | 11 | 10 | 46 |
| HCC-nonBC | 26 | 25 | 40 | 49 | 75 | 215 |

Gender: 2000-2005 vs. 1981-1985 p=0.0003; 2000-2005 vs. 1986-1990 p≤0.0001; 2000-2005 vs. 1991-1995 p=0.1330; 2000-2005 vs. 1996-2000 p=0.0197. Age: 2000-2005 vs. 1981-1985 p≤0.0001; 2000-2005 vs. 1986-1990 p≤0.0001; 2000-2005 vs. 1991-1995 p≤0.0001 and 2000-2005 vs. 1996-2000 p=0.0292. IQR, interquartile range.

Laboratories) and HCV-RNA detected by polymerase chain reaction (PCR), whereas diagnosis of chronic HBV infection was based on the presence of hepatitis B surface antigen (HBsAg) (enzyme-linked immunosorbent assay; Abbott Laboratories).

*Statistical analysis*. The data were analyzed by the Mann-Whitney test for the continuous ordinal data between two qualitative variables. The standard deviation was calculated based on the binomial model for the response proportion. P<0.05 was considered statistically significant.

## Results

*Clinical features of the studied patients*. A total of 1,807 patients with HCC were diagnosed at our hospital from 1981 to 2005. There were 1,347 male (75%) and 460 female (25%) patients, with a median age of 64 years. The proportion of patients diagnosed as HCC-B (HBV-associated: HBsAg positive, HCVAb negative) was 23% (412 of 1,807), whereas 63% (1,134 of 1,807) had HCC-C (HCV-associated: HCVAb positive, HBsAg negative) and an additional 3% (46 of 1,807) had HCC associated with both viruses. The remaining 215 patients (12%) showed both of the virus markers negative.

As shown in Table I and Fig. 1, the number and proportion of HCC-B cases decreased from 1986 to 1990 and thereafter stabilized, whereas those of HCC-C reached the peak in the period 1996-2000 and thereafter decreased. The number and proportion of the HCC-nonBC (HBsAg and HCVAb negative) cases continued to increase in the whole period.

*Background features for patients with HCC*. Fig. 2 shows the median age at diagnosis of HCC-B, HCC-C and HCC-nonBC in five-year intervals (1981-1985, 1986-1990, 1991-1995, 1996-2000 and 2001-2005). The median age of patients at diagnosis of HCC-C showed a steadily significant increase from 58 to 69 years of age during the period. The median age of patients with HCC-B and HCC-nonBC did not significantly change during the period.

Fig. 3 shows the age distribution of patients with HCC-B and HCC-C with the five 5-year intervals. There was no difference in the age distribution of patients with HCC-B during these periods. In contrast, HCC-C obviously had a trend to increase in the number of patients aged >65 years.

Table I shows that the male/female ratio of HCC patients decreased from 4.2 in the period 1981-1985 to 2.1 in 2001-2005, indicating clearly the increase of female patients. In analysis of patients in HCC-C, the male/female ratio in the periods 1981-1985, 1986-1990, 1991-1995, 1996-2000 and 2001-2005 were 6.4, 4.8, 2.5, 2.7 and 1.9, respectively (1981-1985 vs. 2001-2005, p≤0.0001) (Table II). The ratio became clearly smaller, indicates an increase in female patients with HCC-C. On the other hand, the male/female ratio of other types of HCC patients did not significantly change during the period.

## Discussion

This was a two major liver center-based study designed to examine the sequential change in the background of HCC patients during the past 25 years, 1981-2005. More than 80% of our patients had chronic HBV or HCV infections. During the observation period, the number and proportion of HCC-B cases decreased in the period 1986-1990 and thereafter reached a plateau, whereas HCC-C reached a peak in the period 1995-2000 and thereafter slightly decreased. On the other hand, the number and the proportion of HCC-nonBC gradually increased in the periods of 1981-1985, 1986-1990, 1991-1995, 1996-2000 and 2001-2005 being 26 (11%), 25 (8%), 40 (11%), 49 (12%) and 75 (16%), respectively. Previous studies from Japan reported that the proportion of HCC-C had been increased and reached a plateau in the

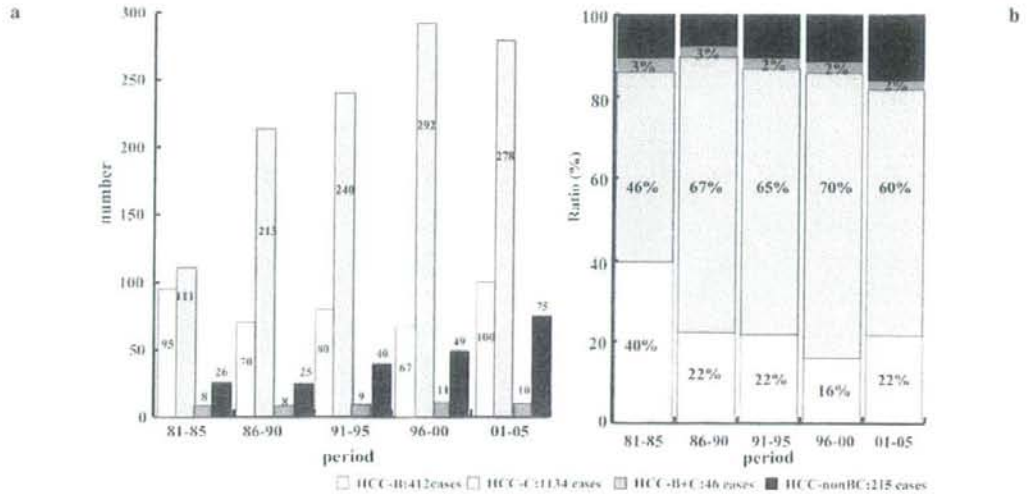


Figure 1. Sequential changes in the number (a) and ratio (b) of HCC patients categorized by etiology during the period 1981-2005 with 5-year intervals.

Table II. The number and male/female ratio of HCC patients during the period of 1981-2005.

| Period | 1981-1985 | 1986-1990 | 1991-1995 | 1996-2000 | 2001-2005 | Total |
|---|---|---|---|---|---|---|
| Number | 240 | 316 | 369 | 419 | 463 | 1807 |
| Total | | | | | | |
| Male | 194 | 257 | 268 | 314 | 314 | 1347 |
| Female | 46 | 59 | 101 | 105 | 149 | 460 |
| Ratio (male/female) | 4.2 | 4.4 | 2.7 | 3.0 | 2.1 | 2.9 |
| HCC-B | | | | | | |
| Male | 69 | 54 | 61 | 55 | 74 | 313 |
| Female | 26 | 16 | 19 | 12 | 26 | 99 |
| Ratio (male/female) | 2.7 | 3.4 | 3.2 | 4.6 | 2.9 | 3.2 |
| HCC-C | | | | | | |
| Male | 96 | 176 | 172 | 212 | 182 | 838 |
| Female | 15 | 37 | 68 | 80 | 96 | 296 |
| Ratio (male/female) | 6.4 | 4.8 | 2.5 | 2.7 | 1.9 | 2.8 |
| HCC-nonBC | | | | | | |
| Male | 21 | 20 | 29 | 40 | 51 | 1347 |
| Female | 5 | 5 | 11 | 9 | 24 | 460 |
| Ratio (male/female) | 4.2 | 4.0 | 2.6 | 4.4 | 2.1 | 2.9 |

HBV and nBnC: NS. HCV: 2000-2005 vs. 1981-1985 p≤0.0001; 2000-2005 vs. 1986-1990 p≤0.0001; 1996-2000 vs. 1981-1985 p=0.0033; 1996-2000 vs. 1986-1990 p=0.0084; 1991-1995 vs. 1981-1985 p=0.0024 and 1991-1995 vs. 1986-1990 p=0.0058.

period of 1981-2001 (8,15,17-19). However, in our study, the number and proportion of HCC-C cases decreased in the period 2001-2005. This may be due to interferon therapy associated with a decreased incidence of HCC (20-24). Iron depletion for chronic hepatitis C patients is a promising modality for lowering the risk of progression to HCC (25,26). Oral supplementation with oral branched-chain amino acids has been useful in the prevention HCC (27). Finally, the chronically HCV-infected population is aging in Japan. Yoshizawa reported that age-specific prevalence for the presence of HCVAb among ~300,000 voluntary blood donors from Hiroshima in 1999 clearly increased with the

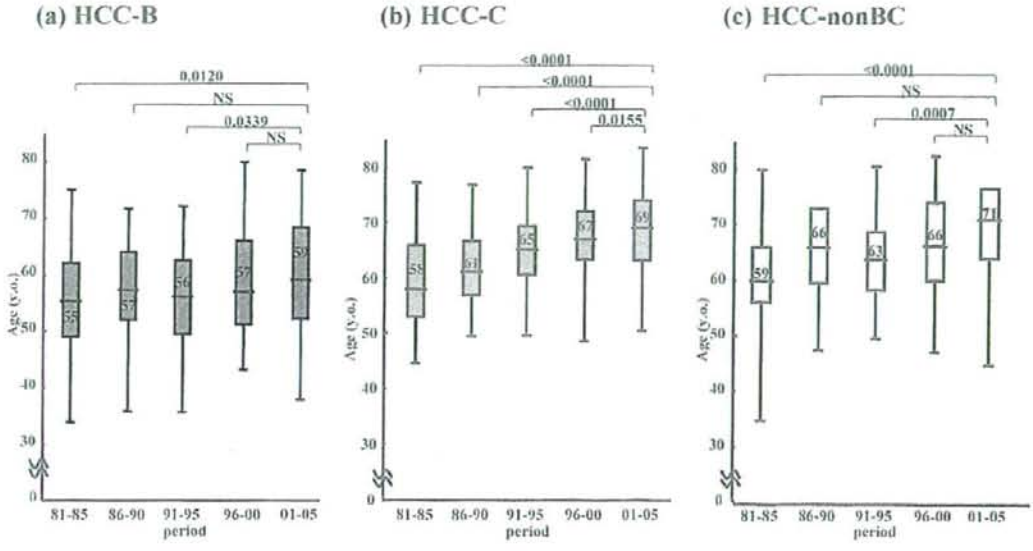


Figure 2. Sequential changes in the median age of HCC patients categorized by etiology during the period, 1981-2005 with 5-year intervals. (a) HCC-B, (b) HCC-C and (c) HCC-nonBC type $p<0.05$.

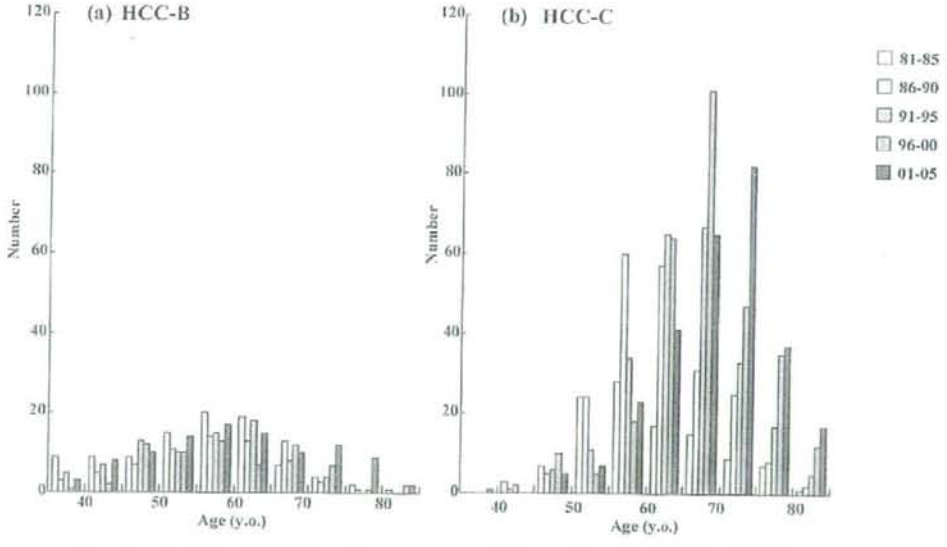


Figure 3. Changes in the age distribution of patients with HCC-B and HCC-C during the period, 1981-2005 with 5-year intervals.

age, reaching the highest proportion of 7% in individuals who were >70 years old (15,16). In this study, the median age of patients with HCC-C steadily increased from 58 to 69 years of age during the studied period. *i.e.* HCV infected people become older and they were regarded as a high risk for HCC.

In almost all populations, males have higher liver cancer proportions than females, with the male/female ratios usually averaging between 2:1 and 4:1 (7). However, the male/female ratio of HCC in Japan was 4.5 in the period 1983-1985 and 2.57 in 2000-2001 (17). In analysis of background features among HCC patients, HCC-B and HCC-nonBC cases revealed no significant change, whereas the male/female ratio of patients with HCC-C steadily decreased from 6.4 to 1.9 during the period. We suggest that the increase of female

patient with HCC-C was caused by the aging of HCV infected people. The increase of females among HCC patients was considered to increase because of HCC-C.

It is known that 2 to 4 decades of chronic HCV infection are required to develop cirrhosis and subsequent HCC (28-31). The number of HCC cases has increased in Japan, because individuals infected with HCV in the past have grown old and have reached the cancer-bearing age. The prevalence of HCV infection in young Japanese individuals is low and the incidence of HCVAb is very low because of preventative actions against HCV infection such as the screening of blood products for HCV and the use of sterile medical equipment (32). Additionally, we showed that the number and proportion of patients with HCC-C cases decreased together with an increase in the median age, whereas the number and ratio of HCC-nonBC steadily increased during the studied period. Based on these findings it may be expected that the incidence of HCC-nonBC in Japan may continue to increase even after the consequence of the HCV epidemic level off in the near future, although Japan is far advanced with regard to HCC-C.

In summary, HCC patients rapidly increased from 1981 to 2000 and this increase originated from HCC-C and the increase of the median age and the number of female patients with HCC-C. Increase in the number and proportion of the HCC-nonBC patients are also significant.

## References


1. El-Serag HB and Mason AC: Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med 160: 3227-3230, 2000.
2. El-Serag HB: Epidemiology of hepatocellular carcinoma. Clin Liver Dis 5: 87-107, 2001.
3. El-Serag HB, Hampel H, Yeh C and Rabeneck L: Extrahepatic manifestations of hepatitis C among United States male veterans. Hepatology 36: 1439-1445, 2002.
4. El-Serag HB: Hepatocellular carcinoma and hepatitis C in the United States. Hepatology 36: S74-S83, 2002.
5. El-Serag HB: Hepatocellular carcinoma: an epidemiologic view. J Clin Gastroenterol 35: S72-S78, 2002.
6. Hassan MM, Frome A, Patt YZ and El-Serag HB: Rising prevalence of hepatitis C virus infection among patients recently diagnosed with hepatocellular carcinoma in the United States. J Clin Gastroenterol 35: 266-269, 2002.
7. El-Serag HB and Rudolph KL: Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 132: 2557-2576, 2007.
8. Kiyosawa K and Tanaka E: Characteristics of hepatocellular carcinoma in Japan. Oncology 62: 5-7, 2002.
9. McGlynn KA, Tsao L, Hsing AW, Devesa SS and Fraumeni JF Jr: International trends and patterns of primary liver cancer. Int J Cancer 94: 290-296, 2001.
10. Bosch FX, Ribes J, Diaz M and Cleries R: Primary liver cancer: worldwide incidence and trends. Gastroenterology 127: S5-S16, 2004.
11. Hamasaki K, Nakata K, Tsutsumi T, *et al*: Changes in the prevalence of hepatitis B and C infection in patients with hepatocellular carcinoma in the Nagasaki Prefecture, Japan. J Med Virol 40: 146-149, 1993.
12. Kato Y, Nakata K, Omagari K, *et al*: Risk of hepatocellular carcinoma in patients with cirrhosis in Japan. Analysis of infectious hepatitis viruses. Cancer 74: 2234-2238, 1994.
13. Shiratori Y, Shiina S, Imamura M, *et al*: Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. Hepatology 22: 1027-1033, 1995.
14. Shiratori Y, Shiina S, Zhang PY, *et al*: Does dual infection by hepatitis B and C viruses play an important role in the pathogenesis of hepatocellular carcinoma in Japan? Cancer 80: 2060-2067, 1997.
15. Kiyosawa K, Umemura T, Ichijo T, *et al*: Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 127: S17-S26, 2004.
16. Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 62: 8-17, 2002.
17. Umemura T and Kiyosawa K: Epidemiology of hepatocellular carcinoma in Japan. Hepatol Res 37 (Suppl 2): S95-S100, 2007.
18. Taura N, Yatsuhashi H, Hamasaki K, *et al*: Increasing hepatitis C virus-associated hepatocellular carcinoma mortality and aging: Long term trends in Japan. Hepatol Res 34: 130-134, 2006.
19. Taura N, Hamasaki K, Nakao K, *et al*: Aging of patients with hepatitis C virus-associated hepatocellular carcinoma: long-term trends in Japan. Oncol Rep 16: 837-843, 2006.
20. Nishiguchi S, Kuroki T, Nakatani S, *et al*: Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 346: 1051-1055, 1995.
21. Nishiguchi S, Shiomi S, Nakatani S, *et al*: Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. Lancet 357: 196-197, 2001.
22. Kasahara A, Hayashi N, Mochizuki K, *et al*: Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology 27: 1394-1402, 1998.
23. Ikeda K, Saitoh S, Arase Y, *et al*: Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: A long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 29: 1124-1130, 1999.
24. Makiyama A, Itoh Y, Kasahara A, *et al*: Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. Cancer 101: 1616-1622, 2004.
25. Kato J, Miyanishi K, Kobune M, *et al*: Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. J Gastroenterol 42: 830-836, 2007.
26. Furutani T, Hino K, Okuda M, *et al*: Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. Gastroenterology 130: 2087-2098, 2006.
27. Muto Y, Sato S, Watanabe A, *et al*: Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol 3: 705-713, 2005.
28. Deuffic S, Poynard T and Valleron AJ: Correlation between hepatitis C virus prevalence and hepatocellular carcinoma mortality in Europe. J Viral Hepat 6: 411-413, 1999.
29. El-Serag HB and Mason AC: Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 340: 745-750, 1999.
30. Planas R, Balleste B, Antonio Alvarez M, *et al*: Natural history of decompensated hepatitis C virus-related cirrhosis. A study of 200 patients. J Hepatol 40: 823-830, 2004.
31. Davila JA, Morgan RO, Shaib Y, McGlynn KA and El-Serag HB: Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 127: 1372-1380, 2004.
32. Sasaki F, Tanaka J, Moriya T, *et al*: Very low incidence rates of community-acquired hepatitis C virus infection in company employees, long-term inpatients, and blood donors in Japan. J Epidemiol 6: 198-203, 1996.

# Interleukin-18 promoter polymorphisms and the disease progression of Hepatitis B virus-related liver disease

KIYOSHI MIGITA, KAZUMI SAWAKAMI-KOBAYASHI, YUMI MAEDA, KAZUHIKO NAKAO, SUSUMU KONDOH, MIKA SUGIURA, RYOKO KAWASUMI, OSAMU SEGAWA, HIDEJI TAJIMA, MASAYUKI MACHIDA, MINORU NAKAMURA, KOJI YANO, SEIGO ABIRU, EIJI KAWASAKI, HIROSHI YATSUHASHI, KATSUMI EGUCHI, and HIROMI ISHIBASHI

OMURA, CHIBA, NAGASAKI, AND IBARAKI, JAPAN

**In this study, we aimed to explore whether interleukin-18 (IL-18) gene-promoter polymorphisms are associated with the outcome of hepatitis B virus (HBV) infection. In all, 204 chronically HBV-infected patients were recruited in this study. Of the 204 HBV-infected patients, 43 were considered to be inactive HBV carriers based on the sustained normalization of serum alanine aminotransferase (ALT) together with seropositivity for the antibody to hepatitis B e-antigen (anti-HBe). A total of 161 patients were found to have chronic progressive liver disease, which included cirrhosis. In these HBV-infected patients, the frequencies of AA genotype of IL-18 gene-promoter polymorphisms at position -607 and C allele at position -137 were significantly higher in inactive HBV carriers compared with those in patients with chronic progressive liver disease. These polymorphisms of the IL-18 promoter regions (-607 and -137) could be associated with different outcomes of HBV infection. (Translational Research 2009;153:91–96)**

**Abbreviation:** ALT = alanine aminotransferase; CI = confidence interval; CPLD = chronic progressive liver disease; anti-HBe = hepatitis B e-antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCC = hepatocellular carcinoma; IFN-γ = interferon-γ; IL-18 = interleukin-18; OR = odds ratio; PCR = polymerase chain reaction; SNP = single nucleotide polymorphism; $Th_1$ = T-helper type 1; TMA = transcription-mediated amplification

Hepatitis B virus (HBV) infection is one of the most prevalent chronic viral diseases in the world.[1] Most individuals with persistent HBV infection develop chronic hepatitis, which can progress to cirrhosis or hepatocellular carcinoma (HCC).[2] Increasing evidence indicates that genetic factors influence the natural history of chronic liver diseases.[3] Furthermore, several recent advances concerning the polymorphism of cytokines that control the host response to the virus could play an important role in determining the outcome of HBV infection.[4] Previous studies have shown that the capacity of cytokine production varies among individuals and correlates with single nucleotide polymorphisms (SNPs) in the promoter region of various cytokine genes.[5] Also, cytokine gene polymorphisms were associated with the severity of the liver disease in patient with HBV infection,[6] which may provide clues to clarify the mechanism for the progression of viral hepatitis.

Interleukin-18 (IL-18) was first described as an interferon-γ (IFN-γ)–producing factor, and it has multiple functions including the activation of cytotoxic T lymphocytes or natural killer cells and the promotion

From the Clinical Research Center, NHO Nagasaki Medical Center, Kubara 2-1001-1, Omura 856-8652, Japan; the Precision System Science Co. Ltd., 88, Kamihongou, Matsudo-shi, Chiba, Japan; the First Department of Internal Medicine, Nagasaki University School of Medicine Sakamoto 1-7-1, Nagasaki 852-8501, Japan; and the National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba-shi, Ibaraki, Japan.

Submitted for publication April 9, 2008; revision submitted November 14, 2008; accepted for publication November 17, 2008.

Reprint requests: Kiyoshi Migita, MD, Clinical Research Center, NHO Nagasaki Medical Center, Kubara 2-1001-1, Omura 856-8652, Japan. e-mail: migita@nmc.hosp.go.jp.

1931-5244/$ – see front matter



doi:10.1016/j.trsl.2008.11.008

## AT A GLANCE COMMENTARY

### Background

The mechanisms underlying pathogenesis and the progression of chronic hepatitis B virus (HBV) infection have not been properly elucidated. Accordingly, no satisfactory therapeutic approaches are available to treat the millions of chronic HBV carriers. Although chronic HBV infection is induced by HBV, the immune system plays an important, yet poorly understood, role in its pathogenesis.

### Translational Significance

In this study, we investigated a relationship between interleukin (IL)-18 promoter polymorphisms and the disease progression of chronic HBV-related liver diseases. Our data suggest that polymorphisms at the IL-18 gene-promoter region could be associated with different outcomes of HBV infection.

of T-helper type 1 ($Th_1$)-type immune responses.[7] Considering these multiple functions, IL-18 may activate effector cells that are involved in the cytotoxicity against pathogens or malignant cells. Because it is involved in the inflammatory cytokine network, IL-18 could have an important role in the development of chronic inflammatory diseases.[8] Recent findings show that the IL-18 gene-promoter region regulates the gene expression of this cytokine.[9] Interestingly, 2 SNPs at position -607 A/C and -137 G/C within the IL-18 promoter region were suggested to alter the IL-18 promoter activity.[9] Taking this evidence into consideration, we investigated the possible role of the SNPs of IL-18 gene-promoter region in the progression of chronic hepatitis B.

## PATIENTS AND METHODS

**Patients.** A total of 204 patients, who were positive for the hepatitis B surface antigen (HBsAg) and who visited the clinics for treatment of liver disease at Nagasaki University Hospital or at National Nagasaki Medical Center between August 1999, and December 2003, were enrolled in this study. Sixty three healthy volunteers (33 males and 30 females) served as a control group. The patients were followed regularly with measurements of serum alanine aminotransferase (ALT) and HBV markers, such as HBsAg, hepatitis B e-antigen (HBeAg), and anti-HBe, using commercially available radioimmunoassay kits (Dainabot, Tokyo, Japan) every 3–6 months. Patients were also examined with ultrasonography or computed tomography of the liver every 3–6 months. Serum HBV-DNA was detected by the transcription-mediated amplification (TMA) method[10] as described previously. The results were expressed as the logarithm of the genome equivalent per milliliter (LGE/mL). The detection limit of this method is 3.7 LGE/mL. The patients receiving IFN therapy or antinucleoside analog reverse transcriptase inhibitor therapy were excluded in this study. The study protocol was approved by the Ethics Committees of both the Nagasaki University Hospital and the National Nagasaki Medical Center, and informed consent was obtained from each individual. Of 204 patients, 43 (20 male, 23 female; mean age ± standard deviation [SD], 55 ± 18 years) were considered to be inactive HBV carriers based on sustained normal serum ALT levels together with seropositivity for anti-HBe throughout the study. Of 43 inactive HBV carriers, all 31 patients tested for HBV-DNA had serum HBV-DNA levels less than $10^5$ copies/mL (Table I). In addition, 161 of the 204 HBV carriers were considered to have chronic progressive liver disease (CPLD) such as chronic hepatitis or cirrhosis, which is manifested by elevated serum ALT levels and by clinical or histologic findings on liver tissue study during the follow-up period. This group comprised 114 males and 47 females (mean age ± SD, 50 ± 14 years). The ratio of males to females was significantly higher in CPLD patients compared with those in inactive HBV carrier ($P = 0.005$) (Table I). In accordance with the previous report, this ratio demonstrated that the patient gender affects the disease progression in HBV infection.[11] The group of patients with CLPD was classified into the following 3 subgroups: (1) 62

**Table I.** Clinical characteristics of 204 HBV carrier

| Variable | Patients with inactive HBV carrier (n = 43) | Patients with CPLD (n = 161) |
|---|---|---|
| Sex (male/female) | 20/23 | 114/47 |
| Age (years) | 55 ± 18 | 50 ± 14 |
| HBeAg/anti-HBe status | 0/43 | 53/109 |
| Chronic hepatitis/cirrhosis | | 68/93 |
| Serum ALT (IU/L) | 21 ± 8 | 74 ± 101 |
| HBV-DNA | (n = 31) | (n = 148) |
| $< 10^5$ copies/mL | 31 | 60 |
| $\geq 10^5$ copies/mL | 0 | 88 |
| Liver biopsy specimen available during study period | (n = 6) | (n = 51) |
| Stage of fibrosis | | |
| F0–F2 | 6 | 15 |
| F3–F4 | 0 | 36 |

*NOTES:* Age and serum ALT are expressed as mean ± SD.

**Table II.** Primers for the SNP analysis

| PCR | SNP | Primer | Sequence |
|---|---|---|---|
| 1st | -607 (A/C)+ | -607ControlF | 5'-CTTTGCTATCATTCCAGGAA-3' |
| | -137 (G/C) | -137R | 5'-AGGAGGGCAAAATGCACTGG-3' |
| 2nd | -607 | -607BtF | 5'-biotin-CTTTGCTATCATTCCADGGAATAGAAAGTTT-3' |
| | | -607BseR | 5'-TGCTGTATCAGATGCAAGCCAGACGGATACCATGAGGAGAATTTTAT-3' |
| 2nd | -137 | -137BtR | 5'-biotin-ACTGCTGTCGGCACTCCTTGGGCCCGC-3' |
| | | -137BseF | 5'-GAGGTACAGGTTTTGGAAGGCACAGAGCCCAACTGAGGAGGAAGAAA-3' |

patients with chronic hepatitis (CH), (2) 52 patients with cirrhosis, and (3) 47 patients with HCC. Of 47 HCC patients, 41 patients (87%) had cirrhosis.

Of 204 HBV carriers, 57 had undergone liver biopsy during the study period, and their degree of liver fibrosis were assessed using the METAVIR system.[12] To ensure a sufficient number of patients in each category, the severity of fibrosis was classified into 2 categories: F0–F2 and F3–F4. Among 57 patients, 36 patients in the CPLD group were classified as category F3–F4. (Table I).

**DNA extraction.** Genomic DNA was isolated from whole blood using the QIAamp DNA blood protocol (Qiagen Ltd., West Sussex, United Kingdom) according to the manufacturer's instructions.

**IL-18 (-607/-137) genotyping.** Two SNPs at position -607 A/C (rs1946518) and -137 C/G (rs187238) in the IL-18 gene promoter were determined by the ligation-mediated genotyping method[13] with a slight modification. A 849-bp fragment that contained the 2 SNPs was amplified by polymerase chain reaction (PCR) using primers -607ControlF and -137R (Table II), LA Taq polymerase (Takara Bio Inc., Shiga, Japan), and approximately 20 ng genomic DNA. The products were subjected to the 2nd PCR to amplify 178-bp and 174-bp fragments for genotyping at -607 and -137 by the primer pairs of -607BtF and -607BseR and of -137BtR and -137BseF, respectively. The second PCR products for the 2 SNPs were mixed and digested by BseRI to generate a 2-base overhang at the SNP sites. The 2 adapters complementary to the 2 possible 2-base overhang generated from a single SNP were prepared by annealing oligonucleotides, one of which was labeled with 2 different fluorescent dyes (FITC/TexasRed for -607C/A or Cy3/Cy5 for -137G/C) and mixed. The BseRI digest was ligated with the mixed adapters by Ligation Convenience Kit (Nippon Gene Co., Ltd., Toyama, Japan) at room temperature (Table III). The biotinylated DNA fragments were bound to Dynabeads M-280 Streptavidin (Dynal Biotech [Invitrogen], Tokyo, Japan) were subjected to the fluorescence measurement after extensive washing. The reactions after the 2nd PCR were automated using MagSNiPer FD (PSS Co., Ltd., Chiba, Japan) equipped with a 12-channel paramagnetic beads handling unit.[14,15]

**Table III.** Oligonucleotides for adapter preparation

| SNP (genotype) | Sequence† |
|---|---|
| -607 (C) | 5'-FITC-TACAAGATTCTGAAGACACCACCCAT CCTTGT-3' |
| -607 (A) | 5'-TexasRed-TACAAGATTCTGAAGACACCACCCA TCCTTTT-3' |
| -137(C) | 5'-Cy3-TACAAGATTCTGAAGACACCACCCATCCT TGA-3' |
| -137(G) | 5'-Cy5-TACAAGATTCTGAAGACACCACCCATCCT TCA-3' |
| Common* | 5'-AAGGATGGGTGGTGTCTTCAGAATCTTGTA -3' |

*The oligonucleotide was annealed to each oligonucleotide above.
†The nucleotides at the position of the SNPs are underlined.

**Statistical analysis.** Results are expressed as mean ± SD. Comparison of the allele and genotype frequencies of different groups were performed using the chi-square test or the Fisher exact test. IL-18 allele frequencies were tested for the Hardy-Weinberg equilibrium for both patients and controls using the Cochrane-Armitage test. The significance level was set at a $P$ value of 0.05.

## RESULTS

We investigated the distribution of IL-18 promoter -137 (C/C) and -607 (A/C) polymorphisms in 204 Japanese HBV-infected patients (case) and 63 healthy volunteers (control). The genotypes at the positions of IL-18 promoter -137 (C/C) and -607 (A/C) polymorphisms were in Hardy-Weinberg equilibrium in both the case subjects and the control subjects (Table IV and Table V).

The genotype frequencies of IL-18 promoter polymorphisms (-607 and -137) in each subgroup of HBV-infected patients are summarized in Table VI. These 204 HBV-infected patients were divided into the 2 groups; 43 patients were considered to be the inactive HBV carrier, and 161 were found to have chronic progressive liver diseases (CPLD). The genotype frequencies of IL-18 promoter polymorphisms (-607 and -137) in these 2 groups of HBV carriers are summarized in Table VII. With regard to the -607 genotypes, 6 (14.0%) inactive HBV carriers had the CC genotypes,

**Table IV.** Frequencies of IL-18 gene-promoter genotypes (-607 and -137) in case subjects

| Locus | Genotype | Observed number (%) | Expected number* | P value† |
|---|---|---|---|---|
| IL-18 -607 | A/A | 55 (27.0) | 64.2 | |
| | A/C | 119 (58.3) | 105.5 | 0.46 |
| | C/C | 30 (14.7) | 39.3 | |
| IL-18 -137 | G/G | 167 (81.9) | 164.1 | |
| | G/G | 32 (15.7) | 37.9 | 0.37 |
| | C/C | 5 (2.5) | 2.2 | |

*Expected phenotype frequencies based on observed allele frequencies and assuming Hardy-Weinberg equilibrium.
†*P* values were calculated using the Cochran-Armitage test for Hardy-Weinberg equilibrium at individual loci.

**Table V.** Frequencies of IL-18 gene promoter genotypes (-607 and -137) in control subjects

| Locus | Genotype | Observed number (%) | Expected number* | P value† |
|---|---|---|---|---|
| IL-18 -607 | A/A | 20 (31.7) | 19.48 | 0.50 |
| | A/C | 30 (47.6) | 31.10 | |
| | C/C | 13 (20.6) | 12.42 | |
| IL-18 -137 | G/G | 52 (82.5) | 51.57 | 0.48 |
| | G/C | 10 (15.9) | 10.68 | |
| | C/C | 1 (1.6) | 0.57 | |

*Expected phenotype frequencies based on observed allele frequencies and assuming Hardy-Weinberg equilibrium.
†*P* values were calculated using the Cochran-Armitage test for Hardy-Weinberg equilibrium at individual loci.

19 (44.2%) had the AC genotype, and 18 (41.9%) had the AA genotype. Of the CPLD group, 24 patients (14.9%) had the CC genotype, 100 (62.1%) had the AC genotype, and 37 (23.0%) had the AA genotype (Table VII). The frequency of the AA genotype was significantly lower in CPLD compared with that in the inactive HBV carriers (odds ratio [OR], 0.41; 95% confidence interval [CI], 0.20–0.84).

Although the frequency of the A allele at position -607 in inactive HBV carriers seems to be higher compared with those of CPLD patients (inactive HBV carrier; 64.0% vs CPLD; 54.0%), no significant difference was found between the inactive HBV carriers and the CPLD groups (Table VIII). However, the frequency of the C allele at position -137 was found to be significantly higher in the inactive HBV-carrier group compared with that in the CPLD group (Table VIII, inactive HBV carrier, 17.4% vs CPLD, 8.4%, $P = 0.024$).

To elucidate the relationship between these polymorphisms and the fibrosis staging, we divided the patients who received liver biopsy into 2 groups by the degree of fibrosis staging (the F0–F2 group, n = 21, and the F3–F4 group, n = 36). No significant difference was

**Table VI.** Genotype frequencies in patients with HBV

| | Patients with HBV | | | |
|---|---|---|---|---|
| Genotype | Inactive HBV carrier (n = 43) (%) | CH (n = 62) (%) | Liver cirrhosis (n = 52) (%) | HCC (n = 47) (%) |
| Locus -607 | | | | |
| CC | 6 (14.0) | 6 (9.7) | 10 (19.2) | 8 (17.0) |
| CA | 19 (44.2) | 42 (67.7) | 32 (61.5) | 26 (55.3) |
| AA | 18 (41.9) | 14 (22.6) | 10 (19.2) | 13 (27.7) |
| Locus -137 | | | | |
| GG | 31 (72.1) | 53 (85.5) | 40 (76.9) | 43( 91.5) |
| GC | 9 (20.9) | 9 (14.5) | 11 (21.2) | 3 (6.4) |
| CC | 3 (7.0) | 0 (0) | 1 (19) | 1 (2.1) |

**Table VII.** The distribution of IL-18 genotype in inactive HBV carriers and CPLD patients

| | Inactive HBV Carrier (n = 43) | | CPLD (n = 161) | | | |
|---|---|---|---|---|---|---|
| | n | % | n | % | OR (95% CI) | P value |
| Genotype -607 | | | | | | |
| A/A | 18 | 41.9 | 37 | 23.0 | 0.41 (.020–0.84) | 0.022 |
| A/C | 19 | 44.2 | 100 | 62.1 | 2.07 (1.05–4.09) | 0.051 |
| C/C | 6 | 14.0 | 24 | 14.9 | 1.08 (0.41–2.84) | >0.999 |
| Genotype -137 | | | | | | |
| G/G | 31 | 72.1 | 136 | 84.5 | 2.11 (0.95–4.65) | 0.099 |
| G/C | 9 | 20.9 | 23 | 14.3 | 0.63 (0.27–1.48) | 0.407 |
| C/C | 3 | 7.0 | 2 | 1.2 | 0.17 (0.03–1.04) | 0.108 |

*NOTES:* P-value: The Fisher exact test.

discovered in IL-18 gene-promoter polymorphisms (-607 or -137) between these 2 groups (Table IX).

## DISCUSSION

The susceptibility to persistent HBV infection is governed by several factors, which include the age at infection. When infection is acquired during the early neonatal period from an HBV-infected mother, only 10% of children will eliminate the virus. In contrast, when infection is acquired during childhood or later, up to 90% will eliminate the virus spontaneously.[16] Twin studies indicate that in addition to the age at infection, the host genetic background influences the outcome of HBV infection.[3] Elimination of HBV infection requires an innate and adaptive humoral and cell-mediated immune response.[17]

Previous studies have shown that the capacity of cytokine production varies among individuals and correlates with the polymorphisms in the promoter region of